Engineering a Novel CDH1 Fluorescent Protein Reporter Construct to Evaluate Cancer Cell Differentiation

Konar, Gregory (School: Southwestern Educational Society)

Visualizing differentiation of cancer cells is a crucial method of measuring cancer progression. A novel fluorescent protein reporter construct was designed with the purpose of recombining to the e-cadherin (CDH1) protein and then being transfected into c8 astrocytes. The construct was created using gene editing software, and it cut into the c-terminus of exon 17 of CDH1 using transcription activator-like effector nucleases (TALENs) and HR. It was hypothesized that the addition of the site-directed nuclease would promote the insertion of the construct into the proper CDH1 locus in c8 astrocytes. The sequence contained a blasticidin selectable marker and a mCherry-SV40 nuclear localization signal (NLS) to aid in visualizing the fluorescent protein in transfected cells. The construct was synthesized and infused into a CDH1 flanking backbone to create a circular plasmid. This was then transfected into c8 cells using a non-liposomal transfection reagent called effectine. The cells were then analyzed for fluorescence using a CCD camera mounted to a brightfield microscope. Software allowed for the visualization of mCherry expressing cells, which were photographed and documented. Sequence verification confirmed the integration of the construct into the proper locus. Since the e-cadherin protein is present in metastatic cancers, this construct can be universally used in all cancer cell lines and could be essential in future evaluations of epithelial cell differentiation in cancer.